Fluoride Vol. 34 No. 3 165-173 2001 Research Report 165

EFFECT OF FLUORIDE ON THYROID FUNCTION AND CEREBELLAR DEVELOPMENT IN MICE

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SUMMARY: The effect of fluoride on murine thyroid function and cerebellar development was studied by administering NaF in drinking water (0.5 g/L) to pregnant and lactating mice, from the 15th day of pregnancy to the 14th day after delivery. Compared to a control group, the NaF-treated pups, at age 14 days, showed a 35% decrease in body weight, a 75% decrease in plasma free T4, and reductions in the cerebellar and cerebral protein concentrations by 27% and 17%, respectively. Consistent histological changes were present in the cerebellum of the treated mice with the external granular layer being markedly reduced or absent, the Purkinje cell bodies being poorly differentiated and arranged in a single layer at the surface of the internal granular layer, and with more apoptotic Purkinje cells being present.

Keywords: Apoptosis, Brain, Cerebellar development, Fluoride-exposed mice, Thyroid function.

INTRODUCTION

Central nervous system development may be disturbed by low or high thyroid hormone levels,[1-3] increased corticosteroid levels,[4] poor nutrition,[5] exposure to X-rays,[6] and chemical agents.[7] Diseases may be caused by a chemical deficiency, *e.g.* Keshan disease (selenium) and endemic goitre (iodine), or chemical toxicity, *e.g.* arsenic poisoning and endemic fluorosis (fluoride). Chronic fluoride toxicity, from fluoride in water, food, or air, is an important public health problem in several countries. Although thyroid function and structure are purported to be unaffected by 1 ppm fluoride in drinking water,[8] adverse changes occur with higher intakes in endemic fluorosis areas or with fluoride treatments. Increased dietary fluoride has resulted in thyroid enlargement,[9] reduced thyroid adenylate cyclase, and decreased blood thyroxine (T4) and triiodothyronine (T3).[10] Yu found a decreased serum T4 and an increased TSH level in the residents of an endemic fluorosis area where the urinary iodine level (162.7±48.7 μg/24 h) suggested an adequate iodine intake.[11]

Thyroid hormones are necessary for maturation in the postnatal animal, particularly for the central and peripheral nervous systems[1,2,12-14] and the skeleton.[15-17] Maturation in the rat cerebellar cortex is markedly affected by thyroid hormone levels. Hypothyroidism and anaemia have occurred not only with antithyroid medications but also with fluoride.[18] Although the effects of fluoride on bone have been studied, there has been little examination

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of its effects upon the developing brain and cerebellum. The aim of this study was to examine the effect of exposure of pregnant and lactating mice to NaF on the thyroid function and cerebellar maturity of their pups during the suckling period.

MATERIALS AND METHODS

Adult Wistar strain mice (Central Pharmacy, Tunisia), weighing about 30 g, were housed at 22±3°C, with light-dark periods of 12 hours, a minimum relative humidity of 40%, free access to water, and a commercial diet (Sico, Sfax, Tunisia) containing 0.720±0.012 µg of iodine/g of diet. After acclimatization to the laboratory conditions for one week the female mice were caged overnight with males and the presence of spermatozoa in the vaginal smear was taken as an indicator of day 0 of pregnancy. The pregnant mice were divided into a control group and a group treated with 0.5 g NaF/L of drinking water from day 15 of pregnancy until the 14th day after delivery. The pregnant mice were allowed to deliver spontaneously three weeks after coitus. At birth the litters were reduced to 8 pups each and the day of birth was considered as postnatal day 0.

The pups of the control and treated mice were sacrificed on postnatal day 14 after anaesthesia with intra-abdominal chloral hydrate. The body weights were measured, and the cerebella and cerebra were weighed and preserved at -20°C until analysed for protein levels or, for some of the cerebella, fixed in bouin solution, embedded in paraffin, serially sectioned at 5 micrometer, and stained with hematoxyline eosin or borated blue toluidine.[19] Brachial artery blood from the pups was centrifuged at 2200 g and the plasma samples kept at -20°C until the free T4 was measured by radioimmunoassay (Cis-Bio kits). After their extraction by the Schmidt and Thannhauser method,[20] slightly modified by Baläzs *et al*,[21] the cerebrum and cerebellar protein contents were assayed according to the method of Lowry *et al*.[22]

Statistical analysis of the means for the treated and the control groups was done with the Mann and Whitney test or Student's (t) test.

RESULTS

Compared with the control group, the 14-day-old mice whose mothers had been treated with NaF, had a 35% decrease in body weight ($p<0.001$) (Figure 1), a 75% decrease in the plasma free T4 level ($p<0.001$) (Figure 2), a 27% decrease in cerebellar protein ($p<0.001$) (Figure 3), and a 17% decrease in cerebral protein ($p<0.001$) (Figure 3), but no significant change in the cerebellar or cerebral weights (Table). Histologically the cerebellum of the control mice showed three layers: the internal granular layer, the molecular layer and the external granular layer. When compared to the controls, the fluoride-affected 14-day-old mice showed a markedly reduced or absent external granular layer, Purkinje cells which were poorly differentiated and

arranged in a single layer at the surface of the internal granular layer, and an increase in apoptotic Purkinje cells (Plate).

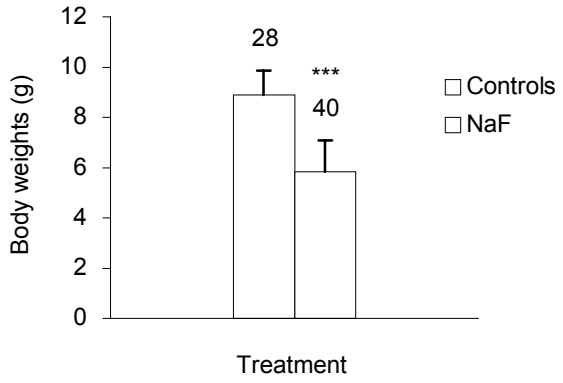

Figure 1. Effect on the body weight of 14-day-old mice administered NaF in the drinking water (0.5g/L) of the mother from the 15th day of pregnancy until sacrifice of the pups on the 14th day after their birth.
Significant differences: Fluoride *vs* Controls ***: p≤0.001.

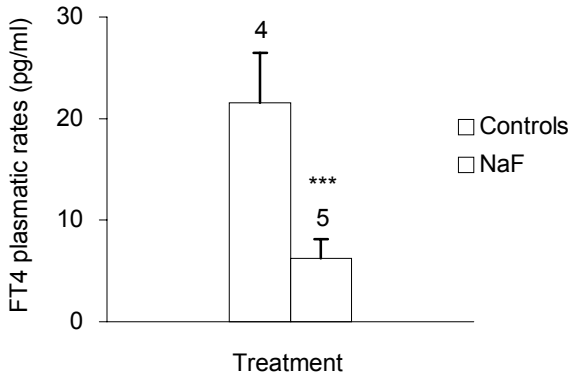

Figure 2. Effect on the plasma thyroxine (free T4) of 14-day-old mice administered NaF in the drinking water (0.5g/L) of the mother from the 15th day of pregnancy until sacrifice of the pups on the 14th day after birth.
Significant differences: Fluoride *vs* Controls ***: p≤0.001.

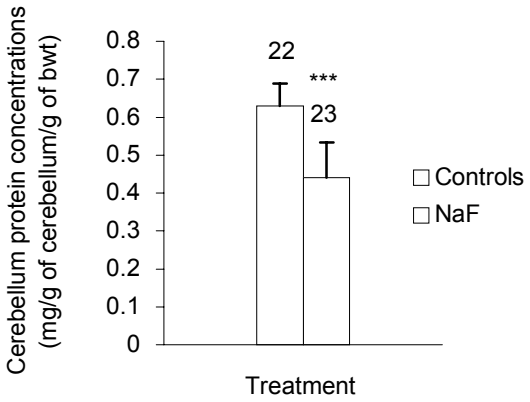

Figure 3. Effect on the cerebral and cerebellar protein concentrations of 14-day-old mice administered NaF in the drinking water (0.5g/L) of the mother from the 15th day of pregnancy until sacrifice of the pups on the 14th day after birth.

Significant differences: Fluoride *vs* Controls ***: p≤0.001

Table. Effect on the weights of the cerebrum and cerebellum of 14-day-old mice administered NaF in the drinking water (0.5g/L) of the mother from the 15th day of pregnancy until sacrifice of the pups on the 14th day after birth

	Cerebrum weight (mg) (mean ± SEM)	Cerebellum weight (mg) (mean ± SEM)
Controls (n=24)	275.66 ± 2.79	86.47 ± 0.90
NaF group (n=28)	233.53 ± 5.97*	78.47 ± 1.65*

*Not significant compared to controls.

Plate. Histological cerebellum sections of 14-day-old mice, controls (A) and treated mice (B) administered NaF in the mother's drinking water (0.5 g/L) from the 15th day of pregnancy until sacrifice of the pups on the 14th day after their birth.

Optical microscopy. HE (X400). The arrows indicate apoptotic Purkinje cells.

DISCUSSION

The decrease in the body weight of the pups affected by fluoride is consistent with earlier findings in rats and mice and results from the reduced free thyroxine and the consequent reduced augmentation by thyroxine of the effect on growth of growth hormone (GH).[23,24] A tendency to increased iodine uptake, reduced T3 and an increased TSH were found by Bachinski *et al* in people living in a high fluoride area, with 122±5 μmol /L (about 2.3 ppm) in the water, compared to those in a control area with 52±5 μmol/L (about 1.0 ppm). Decreases in T3 and T4 have been found after the administration of fluoride to animals,[26,27] including in a study by Yu[11] where 50 ppm of fluoride in the drinking water of rats reduced the serum T3 and T4.

Thyroid deficiency in early life has a marked influence on the functional development of the central nervous system and is accompanied by significant effects on the structural and biochemical maturation of the cerebellum. In our study the administration of fluoride resulted in decreased cerebellar and cerebral protein levels. Previous work has shown that most of the cells in the cerebellum are formed after birth.[3,14,28] Altman considered that different cerebellar interneurones and glial cells appeared in a definite chronological order after birth.[29] Different factors, such as fluoride, might be able to affect cell proliferation in the external germinal layer of the cerebellum. The destruction, by fluoride, of the external granular layer, in the present study, could be explained by the neurones of the external granular layer having already migrated towards the molecular and internal granular layers before the onset of the fluoride treatment and the mitosis of these cells then being stopped by the fluoride.

Similar perturbations have been found in the cerebellum of rats treated with methyl azoxymethanol (MAM) an antimitotic drug which affects the histological[30] and biochemical[31] maturation of the cerebellum of the mouse and rat when given after birth. Rabie *et al* considered a transient reduction in the thickness of the external granular layer of the rat pup cerebellum occurred with the administration of MAM during a limited period of development.[32] In the present study, cerebellar cell formation was depressed during the few days which followed the beginning of the fluoride administration with more apoptotic Purkinje cells appearing. The increase in cell death in the fluoride treated mice might indicate that some granule cells die a certain time after laying down because Purkinje cells were not able to establish contact with them at the proper time.

The increased granule cell deaths in the cerebellum of thyroid deficient animals was related to the suppression of the normal synchronism between the morphogenesis of the Purkinje cell arborizations and the deposition of the granule cells. Purkinje cells might be especially sensitive to lack of thy-

roid hormones, for they are formed earlier and consequently are exposed to thyroid deficiency for a longer time than the other neurones.

Neurotoxicity in cerebellar and neuronally related cell lines can be induced with 3-hydroxykynurenine (3-HK), an endogenous neurotoxin implicated in certain neurodegenerative diseases.[33] At high molecular concentrations of neurotoxin, the toxicity has features characteristic of apoptosis, including chromatin condensation and internucleosomal DNA cleavage.

Cell damage and cell death have been induced by the generation of reactive oxygen species (ROS) through the administration of a wide variety of chemical compounds including fluoride. ROS contribute to neurotoxicity. In this study the external granular layer of the mouse cerebellum was destroyed by fluoride. Other authors found alterations in specific rat brain regions.[34]

In conclusion, we found that ingested fluoride was retained by the cerebellum, interfering with its physiology and inducing neurotoxicity, cell damage, and even cell death.

ACKNOWLEDGEMENTS

The authors are indebted to Mrs Imen Ketata-Hachicha, Nabiha Mezghanni and Houda Trabelsi for their skillful technical assistance. The work was supported by grants from the Direction générale de la Recherche Scientifique et Technique - Tunisie (Appui à la Recherche Universitaire de Base E53/C09).

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